RESEARCH ARTICLE

HLA-DQB1*03 genotype and perioperative blood transfusion are not conducive to the prognosis of patients with gastric cancer

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Background: Gastric cancer (GC) is a disease associated with a higher incidence and mortality, and some host genetic polymorphisms have been reported as potential factors contributing to the development of GC. In view of this, the study was conducted to investigate the effects of *HLA-DQB1* gene polymorphisms and perioperative blood transfusion on prognosis of patients with gastric cancer (GC).

Methods: A total of 142 patients with GC (case group) and 150 healthy controls (control group) were enrolled. Relationship between *HLA-DQB1* gene polymorphisms, perioperative blood transfusion, and clinical pathological parameters of patients with GC after operation was analyzed. Kaplan-Meier curve was applied for analyzing survival rate of patients with GC, and Cox multivariate regression analysis for prognostic factors of patients with GC.

Results: The frequency of *HLA-DQB1**03 gene was increased in patients with GC. Patients with GC with *HLA-DQB1**03 genotype had higher number of tumor size >6 cm, deeper depth of infiltration, higher LNM rate, and later stage of disease. Patients with *HLA-DQB1**03 genotype had lower survival rate compared with other genotypes. Anemia before operation, depth of infiltration in T3 stage and T4 stage, LNM in N1 stage and N2 stage, and *HLA-DQB1**03 genotype were regarded as independent risk factors for patients with GC.

Conclusion: These results demonstrate that *HLA-DQB1**03 genotype and perioperative blood transfusion are not conducive to the prognosis of patients with GC, which could provide a reference for the treatment of GC.

KEYWORDS

gastric cancer, gene polymorphism, *HLA-DQB1*, perioperative blood transfusion, prognosis

1 | INTRODUCTION

Gastric cancer (GC) is known as the fourth most common malignancy around the world with both a higher morbidity and mortality, particularly in Asia (China and Japan).^{1,2} Statistics have revealed that approximately 1 million new cases and 740 thousand deaths would occur in the world annually, and China has the higher incidence with about 400 000 new cases and 300 000 deaths found in each year.³ It is reported that many kinds of risk factors

can affect the development of GC, including family history, age, gender, smoking, food rich in salt or nitro compounds, unhealthy dietary habits, and *Helicobacter pylori* infections.⁴⁻⁶ In the past few decades, many efforts have been made to ameliorate the clinical outcomes of GC, such as early detection, based on nationwide mass screening program and to create treatment strategies, including radical surgery, chemotherapy, and radiotherapy, for improving the prognosis of GC.⁷ Although significant achievements have been made for the early GC treatment, the long-term survival rate

for patients with advanced GC is still unsatisfactory.⁸ Therefore, it is very imperative for us to elucidate the molecular mechanisms regulating the occurrence of GC and the related molecular mechanisms of the disease for creating the targeted therapy for patients with GC.⁹

In the human genome, the human leukocyte antigen (HLA), also deemed as major histocompatibility complex (MHC), is located on chromosome 6 and includes 239 antigenic loci with the length of 3600 kb.^{10,11} It has been suggested that *HLA* genes are reported to modulate the immune system and are inclined to many kinds of autoimmune disorders, especially *HLA-DRB1* and *HLA-DQB1*.^{12,13} Previous studies demonstrated that *HLA-DQB1* gene polymorphisms were correlated with various kinds of cancers, including cervical cancer and GC.^{14,15} Besides, blood transfusions, an important method for saving the life of patients, are often needed during gastrectomy with extended lymphadenectomy.¹⁶ However, transfusion can result in multiple kinds of side effects, including infection, hemolytic transfusion reaction, iron overload, post-transfusal graft vs host disease, as well as polycythemia.¹⁷ The relationship between perioperative blood transfusion and the prognosis of patients with GC remains controversial. Thus, we are intended to investigate the relationship between *HLA-DQB1* gene polymorphisms, perioperative blood transfusion, and patients with GC for providing some references for the treatment of GC.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

This study was approved by the Ethics Committee of Taizhou Hospital of Zhejiang Province, and in strict accordance with the principle of informed consent, which was signed by patients and their families.

2.2 | Study subjects

A total of 142 patients with GC (the case group) underwent gastrectomy with complete follow-up data were enrolled in our study, which were also pathologically diagnosed in Taizhou Hospital of Zhejiang Province between May 2008 and May 2013. Among those 142 patients with GC, 61 cases were female and 81 were males, with the mean age of 62.9 ± 11.2 years old. All relevant data were collected from the record room of Taizhou Hospital of Zhejiang Province. At the same time, 150 healthy people who received physical examination in Taizhou Hospital of Zhejiang Province in the same period were selected as the control group for detection of *HLA-DQB1* gene polymorphisms. Among 150 healthy people, there were 93 males and 57 females with the mean age of 60.1 ± 12.6 years old. The inclusion criteria were as follow: (i) patients had no metastasis of liver, lung, and abdominal cavity before operation; (ii) patients underwent gastrectomy; and (iii) all tumors were epithelial, and nonepithelial tumors such as

sarcoma, mesenchymoma, lymphoma, and endocrine tumors were excluded. The exclusion criteria were as follow: (i) patients with definite genetic relationship; (ii) patients with palliative surgery; (iii) patients who died during perioperative period; (iv) patients had plasma transfusion and other types of blood products during perioperative period; and (v) patients with peritoneal dissemination and distant metastasis during operation. Grading and staging of tumors in strict accordance with the seventh edition of Gastric Cancer TNM staging standards drafted by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC).¹⁸

2.3 | Treatment regimens

All 142 patients with GC were not treated with radiochemotherapy, and gastrectomy was performed after pathological diagnosis. All the subjects were collected in the early morning before operation to study the gene polymorphism. Perioperative period in this study means a period of time from patients with GC received operation to basic recovery, and perioperative blood transfusion was mainly determined by the attending physician according to the patient's condition, and there was no strict system to regulate the critical value of blood transfusion.

2.4 | Extraction of DNA from peripheral blood

Peripheral venous blood (5 mL) was collected from all study subjects via vacuum blood collection tube in the fasting morning and centrifuged at 1610 g for 10 minutes. The DNA was extracted via DNA extraction kit (Takara Holdings Inc., Kyoto, Japan). A total of 500 μ L blood sample and 500 μ L cell lysate were mixed evenly and oscillated for 10 seconds, followed by centrifugation at 25 764 g for 5 minutes to discard the supernatant. After that, 500 μ L cell lysate was added into and supernatant was discarded to leave a little nuclear precipitation. Then, 250 μ L buffer solution was added and mixed evenly for further use. The water bath was conducted for samples at 55°C for rendering the solution transparent. After that, the absolute ethyl alcohol (100 μ L) was added for mixing evenly. The flocculent precipitation and above solution were added into an adsorbing column CB3 for centrifugation at 25 764 g for 60 seconds. The discard solution was discarded with the addition of 600 μ L rinse solution, followed by the centrifugation at 25 764 g for 60 seconds. After the removal of discard solution, the adsorbing column CB3 was stewed at room temperature for 10 minutes. The remaining rinse solution was dried, and the adsorbing column CB3 was placed at clean centrifuge tubes, followed by the addition of 100 μ L buffer solution. After that, the adsorbing column CB3 was placed at room temperature for 10 minutes and centrifuged at 25 764 g for 5 minutes. The solution in the tube was the sample DNA. The purity of DNA was detected at optical density_{260 nm} (OD_{260 nm}) by DNA concentration detector (NANODROP 2000, Thermo Fisher Scientific, California, USA) with the concentration of 100 ± 20 ng/L, and

TABLE 1 Primer sequence of different sites in *HLA-DQB1* gene

Genetic site	Forward	Reverse	Length (bp)
<i>HLA-DQB1*02</i>	F-5'-GTGCGTATTGTGAGCAGAAG-3'	R-5'-GCAAGGTCGTGCGGAGCT-3'	205
<i>HLA-DQB1*03</i>	F-5'-GACGGAGCGCGTGCGTTA-3'	R-5'-AGTACTCGGCGTCAGGCG-3'	122
<i>HLA-DQB1*04</i>	F-5'-CACCAACGGGACCGAGCG-3'	R-5'-GGTAGTTGTGTCTGCATACG-3	200
<i>HLA-DQB1*05</i>	F-5'-CGGAGCGCGTGCGGGG-3'	R-5'-GCTGTTCCAGTACTCGGCAA-3'	128
<i>HLA-DQB1*06</i>	F-5'-CGTGCGTCTTGTGACCAGAT-3'	R-5'-GCTGTTCCAGTACTCGGCAT-3'	121

$OD_{260\text{ nm}}/OD_{280\text{ nm}}$ ranges from 1.6–1.8. The extracted DNA was stored at -20°C .

2.5 | Polymerase chain reaction-sequence-specific primer (PCR-SSP)

The extracted DNA sample was mixed well with the primers (Table 1) which were coated in a D-Mix tube. A total of 2 μL Taq enzyme was added into the D-Mix tube and mixed evenly. After that, 10 μL solution was took up and added into negative control (NC) well. And then, 30 μL DNA sample was also added into the D-Mix tube and shook for 10 seconds. All the wells were added with the above intermixture (10 μL) except the NC well for conducting PCR amplification via the PE9700 type amplification instrument (PerkinElmer Inc., Waltham, Massachusetts, USA). Amplification procedures were as follows: 10 cycles of 96°C for 130 seconds and 63°C for 60 seconds and 20 cycles of 59°C for 50 seconds and 72°C for 30 seconds, till the end. The amplified product was added into agarose gel with constant voltage of 150 V for 5 minutes. After that, the bands were observed under ultraviolet lamp. To verify the results of PCR-SSP, PCR products were directly sequenced by ABI 3730XL sequencer (ABI Company, Oyster Bay, NY) using the Sanger end termination method.

2.6 | Follow-up

All patients were followed up until May 2017 with the follow-up time of 10–60 months (median follow-up time: 33 months). Patients who died during perioperative period were excluded. Among 142 patients with GC, 135 had the follow-up, with the follow-up rate of 95.1%. The follow-up was performed with outpatient service, hospital visit, letters, and telephone. The overall 5-year survival rate was 39.4%. The first time follow-up was conducted 1 month after operation, the first year after operation was followed up every 3 months, and the follow-up would be conducted once or twice every year afterward.

2.7 | Statistical analysis

The statistical analyses in our study were conducted with SPSS 21.0 (IBM Corp., Armonk, NY, USA). The categorical data were presented as ratio or percentage, while the measurement data were presented as the means and standard deviations (SD). Comparisons between groups were calculated by chi-square test and Fisher's exact probability test. Kaplan-Meier curve and log-rank test were performed to determine the factors that might influence the prognosis, and multivariate analysis was performed by Cox proportional hazards regression model. $P < .05$ was considered as statistically significance.

FIGURE 1 Electrophoretogram for the *HLA-DQB1* (HLA-DQ [05,06]). The results revealed that there were 29 effective wells

Sites	Gene number and sites in case group (N = 142 × 2)	Gene number and sites in case group (N = 150 × 2)	P
HLA-DQB1*02	28 (0.099)	44 (0.147)	.077
HLA-DQB1*03	131 (0.461)	87 (0.290)	<.001
HLA-DQB1*04	21 (0.074)	27 (0.090)	.48
HLA-DQB1*05	32 (0.113)	45 (0.150)	.183
HLA-DQB1*06	72 (0.254)	97 (0.323)	.063

TABLE 2 Distribution of HLA-DQB1 gene frequency in the case and control groups

3 | RESULTS

3.1 | The frequency of HLA-DQB1*03 gene is significantly increased in patients with GC

The reference band was set by PCR-SSP. The results of this experiment can be effectively interpreted by the rules of interpretation

(Figure 1). In the case group, 5 specific loci were found, which were consistent with those of the control group. Compared with the control group, the frequency of HLA-DQB1*03 gene in the case group was significantly increased ($P < .05$), while no such difference was found in gene frequency of the other four sites ($P > .05$) (Table 2). The results revealed that the frequency of HLA-DQB1*03 gene is

FIGURE 2 Sequencing maps of each gene site of HLA-DQB1

TABLE 3 Relationship between *HLA-DQB1* gene polymorphisms and pathological parameters of patients with GC

		Sites				
Clinical data	Cases	HLA-DQB1*02 (%)	HLA-DQB1*03 (%)	HLA-DQB1*04 (%)	HLA-DQB1*05 (%)	HLA-DQB1*06 (%)
Age (years)						
≤60	54	4 (28.57%)	26 (39.39%)	1 (10.00%)	7 (43.75%)	16 (44.44%)
>60	88	10 (71.43%)	40 (60.61%)	9 (90.00%)	9 (56.25%)	20 (55.56%)
P		.443	.755	.058	.617	.359
Gender						
Male	81	8 (57.14%)	39 (59.09%)	4 (40.00%)	10 (62.50%)	20 (55.56%)
Female	61	6 (42.86%)	27 (40.91%)	6 (60.00%)	6 (37.50%)	16 (44.44%)
P		.994	.646	.259	.64	.835
Tumor location						
Upper	20	1 (7.14%)	8 (12.12%)	2 (20.00%)	4 (25.00%)	5 (13.89%)
Middle	45	6 (42.86%)	21 (31.82%)	6 (60.00%)	3 (18.75%)	9 (25.00%)
Lower	72	6 (42.86%)	35 (53.03%)	2 (20.00%)	8 (50.00%)	21 (58.33%)
All stomach	5	1 (7.14%)	2 (3.03%)	0 (0.00%)	1 (6.25%)	1 (2.78%)
P		.591	.907	.147	.414	.726
Tumor size (cm)						
≤6	68	9 (64.29%)	25 (37.88%)	7 (70.00%)	11 (68.75%)	16 (44.44%)
>6	74	5 (35.71%)	41 (62.12%)	3 (30.00%)	5 (31.25%)	20 (55.56%)
P		.196	.026	.147	.076	.632
Residual incisal margin						
No	94	9 (64.29%)	42 (63.64%)	7 (40.00%)	14 (87.50%)	22 (61.11%)
Yes	48	5 (35.71%)	24 (36.36%)	3 (30.00%)	2 (12.50%)	14 (38.89%)
P		.874	.548	.792	.056	.455
Anemia						
No	50	5 (35.71%)	23 (34.85%)	2 (20.00%)	7 (43.75%)	13 (36.11%)
Yes	92	9 (64.29%)	43 (65.15%)	8 (80.00%)	9 (56.25%)	23 (63.89%)
P		.967	.933	.296	.448	.896
Depth of infiltration						
T1	10	2 (14.29%)	5 (7.58%)	1 (10.00%)	1 (6.25%)	3 (8.33%)
T2	45	7 (50.00%)	11 (16.67%)	3 (30.00%)	7 (43.75%)	17 (47.22%)
T3	73	5 (35.71%)	41 (62.12%)	6 (60.00%)	7 (43.75%)	14 (38.89%)
T4	14	0 (0.00%)	9 (13.64%)	0 (0.00%)	1 (6.25%)	2 (5.56%)
P		.228	.001	.776	.743	.132
LNM stage						
N1	48	7 (50.00%)	18 (27.27%)	5 (50.00%)	6 (37.50%)	12 (33.33%)
N2	61	5 (35.71%)	25 (37.88%)	3 (30.00%)	10 (62.50%)	18 (50.00%)
N3	22	1 (7.14%)	15 (22.73%)	1 (10.00%)	0 (0.00%)	5 (13.89%)
N4	11	1 (7.14%)	8 (12.12%)	1 (10.00%)	0 (0.00%)	1 (2.78%)
P		.552	.023	.674	.118	.537
TMN stage						
I	21	4 (28.57%)	6 (9.09%)	3 (30.00%)	3 (18.75%)	5 (13.89%)
II	41	6 (42.86%)	15 (22.73%)	3 (30.00%)	5 (31.25%)	12 (33.33%)
III	61	4 (28.57%)	31 (46.97%)	4 (40.00%)	6 (37.50%)	16 (44.44%)
IV	19	0 (0.00%)	14 (21.21%)	0 (0.00%)	2 (12.50%)	3 (8.33%)
P		.118	.015	.369	.948	.729

GC, gastric cancer; LNM, lymph node metastasis; TNM, tumor node metastasis.

TABLE 4 Relationship between perioperative blood transfusion and clinical pathological parameters of patients with GC

Clinical data	Cases	Transfusion group (n = 79)		Nontransfusion group (n = 63)		P
		n	%	n	%	
Age (years)						
≤60	54	18	22.78	36	57.14	<.001
>60	88	61	77.22	27	42.86	
Gender						
Male	81	46	58.23	35	55.56	.749
Female	61	33	41.77	28	44.44	
Tumor location						
Upper	20	14	17.72	6	9.52	.047
Middle	45	29	36.71	16	25.4	
Lower	72	32	40.51	40	63.49	
All stomach	5	4	5.06	1	1.59	
Tumor size (cm)						
≤6	68	29	36.71	39	61.9	.003
>6	74	50	63.29	24	38.1	
Residual incisional margin						
No	94	40	50.63	54	85.71	<.001
Yes	48	39	49.37	9	14.29	
Anemia						
No	50	10	12.66	40	63.49	<.001
Yes	92	69	87.34	23	36.51	
Depth of infiltration						
T1	12	5	6.33	7	11.11	.003
T2	45	16	20.25	29	46.03	
T3	73	51	64.56	22	36.51	
T4	12	7	8.86	5	6.35	
LNM stage						
N1	48	17	21.52	31	49.21	.005
N2	61	41	51.9	20	31.75	
N3	22	13	16.46	9	14.29	
N4	11	8	10.13	3	4.76	
TMN stage						
I	21	6	7.59	15	23.81	.013
II	41	20	25.32	21	33.33	
III	61	40	50.63	21	33.33	
IV	19	13	16.46	6	9.52	

GC, gastric cancer; LNM, lymph node metastasis; TMN, tumor node metastasis.

significantly increased in patients with GC. To verify the PCR-SSP results, the PCR products of five gene sites were sequenced directly, and the results of sequencing were consistent with those of PCR-SSP. The sequence results of each gene site of HLA-DQB1 are shown in Figure 2.

3.2 | Patients with GC with HLA-DQB1*03 genotype have higher number of tumor size >6 cm, deeper depth of infiltration, higher LNM rate, and later stage of disease

As shown in Table 3, in HLA-DQB1*03 genotype, the number of patients with tumor size >6 cm was significantly higher than that of tumor size ≤6 cm ($P < .05$). Patients with HLA-DQB1*03 genotype had deeper depth of infiltration, higher lymph node metastasis (LNM) rate, and later stage of disease (all $P < .05$). No such significant difference was found in gender, age, tumor location, residual incisional margin, and anemia (all $P > .05$). There were no significant differences in all parameters among other genotypes (all $P > .05$). It can be concluded that patients with GC with HLA-DQB1*03 genotype had higher number of tumor size >6 cm, deeper depth of infiltration, higher LNM rate, and later stage of disease.

3.3 | The transfusion group has more patients with age >60 years, with tumors in upper and middle parts, with residual incisional margin, and with anemia before operation, deeper depth of infiltration, higher LNM rate, and later stage of disease

Among 142 patients with GC, 55.60% (79/142) patients need transfusion and 44.40% (63/142) patients do not need transfusion; 55.90% (44/79) patients need more than 4 U transfusion, 30.70% (24/79) patients need 3–4 U transfusion, and 13.4% (11/79) patients need less than 2 U transfusion. Patients were then divided into transfusion ($n = 79$) and nontransfusion ($n = 63$) groups according to the need of perioperative blood transfusion. The transfusion group had more patients with age >60 years, with tumors in upper and middle parts, with residual incisional margin, and with anemia before operation when compared with the nontransfusion group (all $P < .05$). In the transfusion group, deeper depth of infiltration, higher LNM rate, and later stage of disease were found when compared with the nontransfusion group (all $P < .05$). No significant difference was found in gender between the two groups ($P > .05$) (Table 4). The aforementioned results suggested that the transfusion group has more patients with age >60 years, with tumors in upper and middle parts, with residual incisional margin, and with anemia before operation, deeper depth of infiltration, higher LNM rate, and later stage of disease.

3.4 | Perioperative blood transfusion and HLA-DQB1*03 gene polymorphisms are important factors influencing survival of patients with GC

The 5-year survival rate of 142 patients with GC was 39.4%. Patients in the nontransfusion group had the 5-year survival rate of 61.9%, and patients in the transfusion group had the 5-year survival rate of 21.5% (both $P < .05$) (Figure 3A). Patients with HLA-DQB1*03 genotype had lower 5-year survival rate when compared

FIGURE 3 The 5-year survival rates of patients with GC detected by Kaplan-Meier curve. Note: A, the 5-year survival rate of patients in the transfusion and nontransfusion groups; B, the 5-year survival rate of patients with *HLA-DQB1*03* genotype and other genotypes; GC: gastric cancer

with the other genotypes (all $P < .05$) (Figure 3B), suggesting that perioperative blood transfusion and *HLA-DQB1* gene polymorphisms were important factors influencing survival of patients with GC.

3.5 | Anemia before operation, depth of infiltration in T3 stage and T4 stage, LNM in N1 stage and N2 stage, and *HLA-DQB1*03* genotype are regarded as independent risk factors for patient with GC

Multivariate analysis was conducted with gender, age, tumor size, tumor location, anemia, residual incisional margin, depth of infiltration, LNM, TNM stage, perioperative blood transfusion, and *HLA-DQB1* gene polymorphisms enrolled. The results showed that the degree of tumor infiltration, degree of LNM, perioperative blood transfusion, and *HLA-DQB1* gene polymorphisms were related to the prognosis of patients with GC. Anemia before operation, depth of infiltration in T3 stage and T4 stage, LNM in N1 stage and N2 stage, and *HLA-DQB1*03* genotype was regarded as independent risk factors for patients with GC (Table 5).

4 | DISCUSSION

From previous study, GC is associated with a higher incidence and mortality, which need efficacy treatment regimens.¹⁹ Recently, some host genetic polymorphisms have been reported as potential factors resulting in the development of GC.^{20,21} From all above, we conducted this study to investigate the effects of *HLA-DQB1* gene polymorphisms and perioperative blood transfusion on prognosis of patients with GC. Finally, our study demonstrated that *HLA-DQB1*03* genotype and perioperative blood transfusion are not conducive to the prognosis of patients with GC.

TABLE 5 The relationship between indexes and prognosis of patients with GC detected by multivariate analysis

Risk factors	Exp (B)	95% CI	P
Anemia before operation (No/Yes)	2.065	1.14-3.73	.016
Depth of infiltration (T1, T2/T3, and T4)	1.897	1.12-3.21	.017
LNM stage (N1, N2/N3, and N4)	1.916	1.09-3.37	.024
<i>HLA-DQB1</i> genotype (02, 04, 05, and 06/*03)	1.906	1.17-3.10	.009

GC, gastric cancer; LNM, lymph node metastasis; TNM, tumor node metastasis; CI, confidence interval.

First of all, we focused on the *HLA-DQB1* gene polymorphisms and pathological parameters in patients with GC, the results found that, in *HLA-DQB1*03* genotype, the number of patients with tumor size >6 cm was significantly higher than that of tumor size ≤ 6 cm; and patients with *HLA-DQB1*03* genotype had deeper depth of infiltration, higher LNM rate, and later stage of disease. HLAs participate in host immunity as they involve in showing extracellular as well as intracellular antigens to competent immune cells.²² Abnormal expression of HLA has been proved to play important roles in the development of malignancies.²³ It was found in a previous study that both *HLA-DQA1* and *HLA-DQB1* genes have a minor role in *H. pylori* infection and GC.²⁴ And, *HLA-DQ* types were also proved to influence the progression to atrophy in *H. pylori* infection.^{25,26} Wu et al drew the conclusion that *HLA-DQB1* alleles are associated with susceptibility or resistance to GC and also influence its clinical features.²⁷ Besides, it has been reported by previous researcher that *HLA-DQ* locus may play a different role in the development of *H. pylori*-related chronic gastritis and diffuse-type gastric adenocarcinoma.²⁸

Additionally, our study clarified that the transfusion group had more patients with age >60 years, with tumors in upper and middle parts, with residual incisional margin and with anemia before operation when compared with the nontransfusion group. In the transfusion group, deeper depth of infiltration, higher LNM rate, and later stage of disease were found when compared with the nontransfusion group. Studies focused on perioperative blood transfusion and cancers confirmed that transfusions may raise the frequency and earlier recurrences of solid tumors.^{29,30} It is reported that patients with GC were more likely to need blood transfusion with higher age, female gender, surgical extension to adjacent organs/structures, and tumor location.³¹ As reflected in a previous study, red blood cell transfusions (RBCTs) may affect postoperative recovery because it would contribute to the risk of transfusion-related immunomodulation, and RBCTs are related to worse postoperative short-term outcomes in patients with GC.³² In line with our study, a direct negative correlation was found between perioperative blood transfusion and prognosis in patients receiving GC surgery.¹⁷ The cancer-related anemia, a multifactorial disease, can cause chronic blood loss, metabolite homeostasis, and immune response.³³ Patients with preoperative anemia had poor outcomes, which can be improved by frequently required blood transfusion therapy.³⁴

Furthermore, the multivariate analysis demonstrated that anemia before operation, depth of infiltration in T3 stage and T4 stage, LNM in N1 stage and N2 stage, and *HLA-DQB1*03* genotype were regarded as independent risk factors for patients with GC. Anemia is usually known as a negative predictor of response to chemoradiotherapy.³⁵ One study also concluded that patients with preoperative anemia would have poor prognosis,³⁶ which was in line with our study. Previous evidence conducted by Kulig J et al also demonstrated that depth of infiltration, LNM, and distant metastases, and residual tumor category were the independent prognostic factors for patients with GC.³⁷ Additionally, consistent with our result, previous study also proved that tumor invasion and LNM were independent prognostic factors for patients with GC.³⁸ *HLA-DQ* locus has also been reported to be correlated with the development of diffuse-type gastric adenocarcinoma.²⁸

In conclusion, our preliminary study demonstrated that *HLA-DQB1*03* genotype and perioperative blood transfusion contribute to poor prognosis of patients with GC. Our conclusion provides a reference for the clinical treatment of patients with GC. But due to our limited sample size, our results need further confirmation by a large-scale study.

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